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Effect of wilting and additives on fatty acid composition of red clover silage

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Introduction Forage lipids are typically rich in polyunsaturated fatty acids (PUFA) 18:2n-6 and 18:3n-3, but conservation method greatly affects lipid content and composition. Wilting of grass decreases the proportion of PUFA in forage whereas harvesting at early growth stage and using formic acid to restrict fermentation in silo increases their proportions (Dewhurst et al. 2006). However, the effect of wilting and extent of in silo fermentation on fatty acids in red clover (*Trifolium pratense*) is less studied. It was hypothesized that 1) wilting decreases PUFA content of red clover and 2) restricting microbial fermentation in silo by formic acid preserves fatty acids better than inoculation with lactic acid bacteria.

Material and Methods Pure red clover herbage (cultivar Saija, Boreal Plant Breeding Ltd) was grown in Finland (Jokioinen 60˚49'N, 23˚28'E) and cut at early flowering. One part of the cut herbage was ensiled immediately and other part was wilted to two higher dry matter (DM) contents. The DM content of ensiled material was 194, 358 and 522 g/kg for 0, 24 and 48 h of wilting, respectively. Additive treatments were as follows: untreated, formic acid (4 L pure formic acid per tn herbage) or lactic acid bacteria (*Lactobacillus plantarum* 10^6 colony-forming units (cfu)/g herbage, Lactofast, Kemira Ltd). The amount of added liquid (water plus additive) in herbage was the same (10 ml/kg) for all treatments. The herbage was compacted into minisilos in triplicate (958, 875 and 708 kg/m^3 for 0, 24 and 48 h of wilting, respectively). The 120 ml minisilos were of glass and sealed with a rubber stopper and a plastic screw cap. They were opened 191 days after ensiling. Chemical composition of forages was determined as described earlier by Koivunen et al. (2015) with exception of lipid extraction and fatty acid methylation that was according to Halmemies-Beauchet-Filleau et al. (2013). Results were analyzed by ANOVA using the Mixed procedure of SAS (SAS 9.3, Institute Inc., Cary, NC). Sums of squares for treatment effects were further separated into single degree of freedom comparisons using orthogonal contrasts.

Table 1. Dry matter content (g/kg) and fermentation quality (g/kg dry matter) of red clover silages

Wilting time, h	Additive	Dry matter	pH	Sugars	Lactic acid	Acetic acid	Butyric acid	Ammonia-N, g/kg N
0	No	213	4.07	12	114	33	2.9	30.4
	Formic acid	215	4.03	19	53	33	5.3	16.0
	Lactic acid bacteria	217	4.07	28	113	27	3.3	21.6
24	No	387	4.31	41	94	30	1.2	33.7
	Formic acid	391	4.26	27	53	32	1.6	17.4
	Lactic acid bacteria	386	4.21	52	89	15	1.7	17.9
48	No	534	5.11	115	30	17	0.8	24.4
	Formic acid	534	4.67	134	16	14	1.1	18.2
	Lactic acid bacteria	530	4.36	88	56	11	0.9	18.6
SEM		3.2	0.028	4.8	1.4	2.7	0.49	1.59
Statistical significance								
Wilting, 0 vs 24 and 48 h (1)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.40
Length of wilting, 24 vs 48 h (2)		<0.01	<0.01	<0.01	<0.01	<0.01	0.16	0.06
No additive vs. additives (3)		0.83	<0.01	0.57	<0.01	0.03	0.08	<0.01
Formic acid vs. lactic acid bacteria (4)		0.39	<0.01	0.27	<0.01	<0.01	0.09	0.11
Interaction 1x3		0.53	<0.01	0.07	<0.01	0.46	0.17	0.81
Interaction 1x4		0.21	<0.01	0.03	<0.01	0.38	0.04	0.08
Interaction 2x3		0.54	<0.01	0.70	<0.01	0.65	0.82	<0.01
Interaction 2x4		0.93	<0.01	<0.01	0.09	0.02	0.80	0.97

Results and Discussion Wilting improved the fermentation quality of all silages by decreasing (P<0.01) the concentration of acetic and butyric acids (Table 1). Forage ensiled with formic acid was the most restrictively fermented one. The fatty acid content of ensiled material decreased gradually as the wilting time prolonged (Table 2). The proportion of 18:3n-3 decreased markedly during wilting from 50 to 43 g per 100 g fatty acids, whereas that of 18:2n-6 remained constant. The fatty acid content of silages was slightly higher than that of ensiled material (Table 2). This is probably due to fermentative losses of carbohydrates and proteins in silo as well as lipid synthesis by microbes. Changes in silage fatty acid composition due to additive treatment and extent of fermentation in silo were in general marginal. Nevertheless, using formic acid slightly decreased the proportion of 18:3n-3 and increased that of 18:2n-6 relative to lactic acid inoculum (P<0.04, interaction) in silages prepared without wilting. In addition, using lactic acid bacteria in silage preparation increased the proportion of 16:0 and 18:0 inherent to bacterial lipids relative to silage prepared with formic acid (P<0.01).

Table 2. Total fatty acid (FA) content (g/kg DM) and fatty acid composition (g/100 g FA) of ensiled plant material and silages

Wilting time, h	Additive	Total FA	16:0	18:0	18:1n-9	18:2n-6	18:3n-3
Ensiled material							
0	All treatments	15	15	2.0	1.9	25	50
24	All treatments	10	17	2.3	1.7	25	46
48	All treatments	7.9	18	2.5	1.7	24	43
Silages							
0	No	16	18	2.4	1.7	20	54
	Formic acid	17	17	2.2	1.7	22	53
	Lactic acid bacteria	16	17	2.5	1.7	19	55
24	No	12	19	2.6	1.5	21	51
	Formic acid	12	18	2.4	1.5	20	50
	Lactic acid bacteria	11	19	2.6	1.4	19	48
48	No	12	18	2.5	1.7	20	47
	Formic acid	10	19	2.6	1.6	20	47
	Lactic acid bacteria	9.2	20	2.7	1.6	20	46
SEM		0.39	0.33	0.05	0.04	0.5	0.8
Statistical significance for silages							
Wilting, 0 vs 24 and 48 h (1)		<0.01	<0.01	<0.01	<0.01	0.72	<0.01
Length of wilting, 24 vs 48 h (2)		<0.01	0.05	0.36	<0.01	0.94	<0.01
No additive vs. additives (3)		0.03	0.50	0.37	0.05	0.54	0.21
Formic acid vs. lactic acid bacteria (4)		0.02	<0.01	<0.01	0.09	0.02	0.75
Interaction 1x3		0.04	0.16	0.35	0.50	0.09	0.47
Interaction 1x4		0.52	0.11	0.16	0.86	0.04	0.04
Interaction 2x3		0.05	<0.01	0.06	0.28	0.20	0.38
Interaction 2x4		0.68	0.72	0.08	0.68	0.94	0.61

Conclusion Wilting decreases markedly red clover's fatty acid and 18:3n-3 content, whereas the extent of fermentation in silo has marginal effects on fatty acids.

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